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THE THERMAL DEATH POINT AND LIMITING HYDROGEN ION CONCENTRATION OF PATHOGENIC STREPTOCOCCI

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Since the epidemic of septic sore throat in Boston in 1911 the thermal death point of streptococci has acquired an added importance, as that epidemic led to the belief that a streptococcus was the causative agent of the disease. Added responsibility was placed, therefore, on the process of pasteurization as a protective agent against septic sore throat.

Previous to the work of two of us[1] in 1910, it was a generally accepted fact that streptococci were destroyed at temperatures considerably lower than those commonly used in pasteurization. One strain of streptococcus was found the thermal death point of which was 75.6 C. (168 F.) when heated for 30 minutes, and many other streptococci which were able to resist a temperature of 65.6 C. (150 F.) for a period of 30 minutes. This gave a new aspect to the subject of the thermal death point of streptococci and led us[2] to further experiment along this line which showed that there was a wide variation in the thermal death point of 139 cultures of streptococci isolated from cow feces, milk and cream. When heated in milk under conditions similar to pasteurization at 60 C. (140 F.) the lowest pasteurizing temperature, 64.03% of cultures survived. At 62.8 C. (145 F.) the usual temperature for pasteurization, 33.07% of cultures survived. At 71.1 C. (160 F.) 2.58% survived and all were destroyed at 73.9 C. (165 F.). In all experiments the milk was heated, then held for 30 minutes. Davis[3] has also found that 20 of 74 strains of hemolytic streptococci from milk, and having practically no virulence, were destroyed when heated at 68.3 C. (155 F.) for 30 minutes.

Since it has been fairly definitely decided that the streptococci are the causative agents of septic sore throat the ability of certain of this

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[1] Ayers, S. H., and Johnson, W. T., Jr.: U. S. Dept. Agri., Bur. Anim. Indust., 1910, Bull. 126.

[2] Ayers, S. H., and Johnson, W. T., Jr.: Jour. Agr. Research, 1914, 2, p. 321.

[3] Am. Jour. Pub. Health, 1918, 8, p. 40.

group of organisms to stand temperatures above that of pasteurization naturally presents a grave situation. If pathogenic streptococci are able to survive the usual process of pasteurization then the value of the process, from a sanitary standpoint, is materially lowered.

Experience with the use of properly pasteurized milk and the determination of the thermal death point of pathogenic streptococci by various investigators, indicate very clearly, however, that the thermal death point of these organisms is relatively low and that they are readily destroyed by proper pasteurization. Thus, Hamburger,[4] who studied the epidemic of septic sore throat in Baltimore in 1912, traced this epidemic to a certain milk supply. Advice was given to boil all milk and the dairy connected with the epidemic raised the temperature of their flash pasteurization to 71.1 C. (160 F.), then changed to the holder process where the milk was heated to 62.8 C. (145 F.) and held for a period of 30 minutes. The cases of sore throat that followed were neither so severe or so numerous and did not follow the milk supply but appeared to have been transmitted from individual to individual. He[5] also found that a streptococcus isolated from a patient having a case of sore throat was killed by heating in milk at 62.8 C, (145 F.) for 30 minutes. Again Capps and Miller,[6] who studied the Chicago epidemic of septic sore throat, traced it to a dairy where the milk was pasteurized by the flash process at 71.1 C. (160 F.). On certain dates they found that there was a pronounced failure to pasteurize and following these dates there were outbreaks of septic sore throat. These authors believed that the final responsibility for the epidemic rested on the inadequate and unreliable pasteurization. They state that the absolute protection of the children of the Michael Reese Hospital from infection by efficient pasteurization demonstrates this point. Bray,[7] who studied an epidemic of tonsillitis of tuberculous patients, traced the epidemic to a milk supply of one farm where a carrier presumably infected the milk. Forty cases of tonsillitis resulted among 400 people. As soon as the epidemic broke out the milk was pasteurized, and from that time only 1 case appeared.

From the results achieved from the proper pasteurization of milk it seems evident that the thermal death point of pathogenic streptococci, which cause septic sore throat, is relatively low. This belief is

[4] Jour. Am. Med. Assn., 1912, 58, p. 1109.

[5] Bull. Johns Hopkins Hosp., 1913, 24, p. 1.

[6] Jour. Am. Med. Assn., 1912, 58, p. 1848.

[7] Ibid., 1915, 64, p. 1127.

borne out by the results of the studies of Davis,[3] who found that streptococci isolated from cases of sore throat were readily killed by heating at 60 C. (140 F.) for 30 minutes. Davis[8] also found that none of 24 strains of pathogenic hemolytic streptococci of human origin resisted heating at 60 C. (140 F.) for 30 minutes. He makes the following statement: "I know of no evidence that strains of streptococci pathogenic to man can resist the usual temperature of pasteurization, 145 F., for 30 minutes."

In this paper it is desired to present further results bearing on the thermal death point of pathogenic streptococci. Thirty-five cultures of supposedly pathogenic streptococci were obtained from various sources. Some of these were sent to us in pure cultures after having been isolated from various cases of infection and others were isolated in our laboratory from pus and body fluids. All cultures were tested for pathogenicity by intravenous inoculation of rabbits. The hemolytic power was determined on blood-agar plates incubated for 24 hours. Fermentation tests were made and the thermal death point determined by inoculation of tubes of milk with several drops of fresh broth culture. These tubes were then heated at different temperatures for a period of 30 minutes in order to duplicate the process of pasteurization.

The results of this work are shown in Table 1. It will be noted from the table that all of the A series cultures were hemolytic. Many of them produced infections and often death in rabbits and all had a thermal death point at 60 C. (140 F.) or lower. In fact, only 5 showed a thermal death point as high as 60 C. (140 F.); 11 were destroyed at 57.2 C. (135 F); 6 at 54.5 C. (130 F.); and 5 at 51.7 C. (125 F.) out of a total of 27 cultures. Our results indicate, therefore, that pathogenic streptococci may be destroyed in milk by pasteurization at 60 C. (145 F.) for 30 minutes.

Further reference to the table shows 8 cultures in the B series, the source of which seemed to indicate that they might be pathogenic. Most of these cultures, however, were isolated from pus or body fluids, and it is quite possible that nonpathogenic streptococci were associated with the pathogenic strains and were obtained on plating instead of the more difficultly isolated pathogenic types.

It will be seen that none of the B series were pathogenic to animals. Six of the 8 cultures were not hemolytic and 1 of the other 2 only slightly so. Most of them had a high thermal death point. The most interesting point in connection with the B series of cultures was the difference in the limiting hydrogen ion concentration, in a dextrose-yeast-peptone medium. The P_H limit of the A series ranged from 5.4-6.0 and in the B series from 4.5-4.7.

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[8] Jour. Am. Med. Assn., 1912, 58, p. 1852.

TABLE 1

RESULTS OF A STUDY OF CULTURES FROM PATHOLOGIC SOURCES

No.	Source	Results of Rabbit Inoculation	Hemolysis	Dextrose Yeast-Peptone Broth P_H	Thermal Death Point	
					°C.	°F.
A1	Puerperal infection	Joint enlargement, cultures recovered	+	5.7	60.0	140
A2	Puerperal infection	Death, cultures recovered...	+	5.6	54.5	130
A3	Peritonitis	No effect	+	5.6	54.5	130
A4	Otitis	Partial paralysis hind legs.	+	6.0	57.2	135
A5	Tonsil	Lost	Lost	Lost	57.2	135
A6	Blood, meningitis	No effect	+ sl.	5.6	57.2	135
A7	Renal infection	No effect	+ sl.	5.6	57.2	135
A9	Septicemia, phlebitis	No effect	+	5.8	57.2	135
A11	Blood, death from gas bacillus infection	No effect	+ sl	5.5	57.2	135
A17	Cervical abscess	No effect	+ sl.	5.5	60.0	140
A18	Necropsy, terminal infection by streptococcus septicemia	No effect	+ sl.	5.6	57.2	135
A19	Abscess in guinea-pig inoculated with B. mallei	Died, culture recovered	+	5.4	57.2	135
A22	S. pyogenes, Cornell Vet. College	Died, kidney and liver congested	+	5.6	54.5	130
A23	Sinus pus	No record	+ sl.	5.5	60.0	140
A24	Knee joint pus	No effect	+	5.8	57.2	135
A26	Knee joint fluid	No effect	+	5.5	60.0	140
A27	S. pyogenes (Rosenberger)	Died	+	6.0	51.7	125
A30	S. pyogenes (Holman)	Sick and recovered	+ sl.	5.5	54.5	130
A31	S. pyogenes (Homan)	Died	+	5.7	57.2	135
A32	Heart blood at necropsy	No effect	+ sl.	5.4	57.2	135
A33	Infected finger	No effect	......	5.5	51.7	125
A34	Infected finger	Sick, noduled in lungs when killed	+	5.4	51.7	125
A35	Pneumonia, guinea-pig	Died	+	5.5	57.2	135
A36	Peritonsillar abscess	Died	+	5.4	54.5	130
A37	Severe sore throat	Partially paralyzed	+	5.6	51.7	125
A38	Severe sore throat	Died	+	5.7	60.0	140
A39	Ear discharge, purulent otitis media	Paralysis of hind legs.	+	5.6	51.7	125
B13	Pleural pus	No effect	—	4.6	68.3	155
B14	Pleural fluid	No effect	+ sl.	4.6	65.6	150
B15	S. endocarditis, from blood	No effect	—	4.7	62.8	145
B20	S. wolfson (Steele)	No effect	—	4.6	62.8	145
B21	S. pyogenes X, Cornell Vet. College	No effect	+	4.6	60.0	140
B25	Elbow joint	No effect	Green	4.6	71.1	160
B28	Pleural pus	No effect	—	4.7	60.0	140
B29	Pus from scrotum	No effect	Green	4.6	71.1	160

The higher limiting hydrogen ion concentration with the B series cultures with the fact that none of them were pathogenic to rabbits, that most of them were not hemolytic, that they grew more vigorously in cultures, and in general they fermented a larger number of test

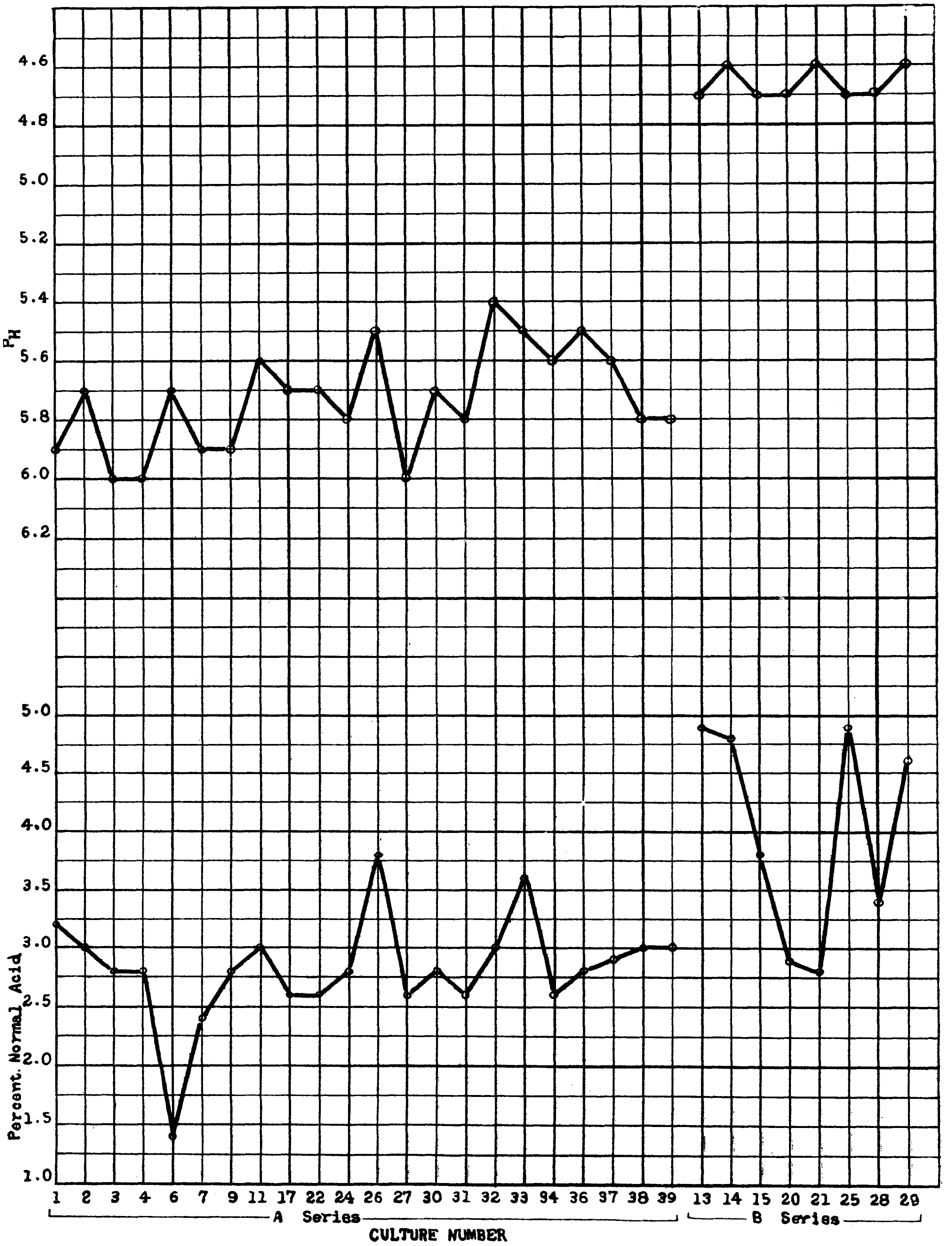

Chart 1.—Acidity by titration and the limiting hydrogen ion concentration of the A and B series.

substances than the A series, seems to separate them sharply from the pathogenic A series cultures. This leads to a further consideration of the limiting hydrogen ion concentration of pathogenic streptococci.

THE LIMITING HYDROGEN ION CONCENTRATION OF PATHOGENIC STREPTOCOCCI

Numerous investigators have found that in general the pathogenic streptococci produce less acid from carbohydrates and other test substances than the nonpathogenic strains. The differences in the amounts of acids formed, however, as shown by titration have not been sufficiently constant to clearly show a difference. Distinct differences in acid-forming ability are, however, clearly shown by means of the hydrogen ion concentration.

In Chart 1 is plotted the per cent of normal acid formed in a dextrose-yeast-peptone medium by cultures of the A and B series, together with the hydrogen ion concentration produced by the same cultures. This medium consisted of 1% dried yeast (a preparation known as cerevisine), 1% peptone and 1% dextrose, with the hydrogen ion concentration adjusted to P_H 7.2.

From the graph it is apparent that titration did not show a constant difference in the amount of acid formed by the A and B series of cultures. While in general the B series show more acid, 4 of the 8 cultures produced a quantity of acid which was within the range of the amounts formed in the A series. A glance at the curves, in the upper part of Chart 1 of the hydrogen ion concentration reached in the same medium, shows a distinct difference in concentration between the A and B series. The P_H values reached by the A series were from 5.4-6.0 while those of the B series were from 4.6-4.7. It is considered that these ranges in P_H values are the limiting hydrogen ion concentration only for the medium used in our experiments. These limits of hydrogen ion concentration might possibly be changed in other mediums with different concentrations of sugar and different buffer actions.

It is believed that the difference in the limiting hydrogen ion concentration between the A series, which is considered to be the pathogenic streptococci, and the B series, which is considered to be the nonpathogenic, represents a distinct differential characteristic. This opinion is strengthened by the figures in Table 2, where it will be seen that of 34 cultures isolated from pathologic sources the limiting hydrogen ion concentration of 26, or 76.5%, ranged from P_H 5.4-6.0 while 8, or 23.5%, showed limiting P_H values from 4.5-5.0.

These results should be compared with cultures obtained from human mouths, from feces, udders and mouths of cows, and milk. Of the 18 cultures from the human mouth all showed a high limiting hydrogen ion concentration. Among the cultures obtained from feces, udders and mouths of cows 82 cultures out of a total of 86, or 95.4%, showed a high limiting hydrogen ion concentration. Among the 60 cultures isolated from milk, 54, or 90%, showed a high limiting hydrogen ion concentration ranging from 4.5-5.0, while 6, or 10%, ranged from 5.4-6.0. The 6 cultures while showing the same limiting hydrogen ion concentrations as the A Series, considered pathogenic streptococci, differered considerably in that they were not hemolytic and in general fermented a much larger number of carbohydrates and other test substances. The limiting hydrogen ion concentrations were obtained in dextrose-yeast-peptone cultures and it is interesting to mention that the same limiting hydrogen ion concentrations were reached in all of the carbohydrates and other test substances, which the organisms were capable of fermenting.

TABLE 2

STREPTOCOCCI GROUPED ACCORDING TO SOURCE AND LIMITING HYDROGEN ION CONCENTRATION

P_H	Pathologic Sources	Human Mouth	Feces, Udders and Mouths of Cows	Milk
4.5-5.0	Hemolysis slight 2 negative 6 8-23.5% B series	Hemolysis — 18-100%	82-95.4%	Hemolysis — 54-90%
5.4-6.0	Hemolysis + 26-76.5% A series	Hemolysis — 0-0%	4-4.6%	Hemolysis — 6-10%
Total	34	18	86	60

The difference in the limiting hydrogen ion concentration among the streptococci has been previously noted.[9] More recently, Smillie[10] found that as a general rule the more pathogenic streptococci produced a lower acidity than the less pathogenic strains. Most of his acidity determinations were made by means of titrations but he determined the hydrogen ion concentration of 5 cultures. The liberty is taken of reproducing a table in revised form from his paper which may be seen in Table 3. This shows the limiting hydrogen ion concentrations reached by 5 cultures, one of which was evidently nonpathogenic. It is of interest to note that the nonpathogenic strain, No. 57, reached a limiting hydrogen ion concentration P_H 4.5, while that of the pathogenic strains averaged around P_H 5.5.

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[9] Ayers, S. H.: Jour. Bacteriol., 1916, I, p. 84.

[10] Jour. Infect. Dis., 1917, 20, p. 45.

In a study of the sugar fermentation Smillie used sugar-free veal infusion broth as a basic medium and it is rather interesting that he obtained two limiting hydrogen ion concentratons, which agree with those obtained by us in a dextrose-yeast-peptone medium.

Although Smillie did not give any particular consideration to the significance of these limiting hydrogen ion concentrations his results, with even a few cultures, strengthen our opinion that the limiting hydrogen ion concentration of streptococci will prove of value and it at least deserves further careful study.

TABLE 3

LIMITING HYDROGEN ION CONCENTRATION OF STREPTOCOCCI AS GIVEN BY SMILLIE

No.	Type of Hemolvsis	Virulence for Animals 24-Hour Culture	Sugar Reactions P_H*							
			Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin
57	Nontypical beta incomplete	0.5 c c did not kill mouse	4.5	4.4	4.6	4.4	7.4	4.7	4.8	4.7
56	Beta	0.5 c c killed mouse in 7 days	5.1	5.5	5.4	5.5	7.2	5.7	7.0	7.2
58	Beta	0.5 c c killed mouse in 6 days	5.4	5.5	5.6	5.6	7.2	5.5	7.2	7.0
59	Beta	0.5 c c killed mouse in 3 days	5.7	5.5	5.6	5.8	7.2	5.7	7.2	7.2
50	Beta	0.5 c c killed mouse in 2 days	5.4	5.5	5.3	5.4	7.0	5.6	7.0	7.0

* Control P_H 7.2.

The value of the determination of hydrogen ion concentration is clearly shown by the results previously discussed and in this connection it is desired to emphasize another point, which is, that the determination of the hydrogen ion concentration gives the limiting value reached in the fermentation regardless of the point at which it starts, provided of course, it is within the growing range of the organism. On the other hand, with titration methods the original reaction may be so high that when the control titration is subtracted from the total titration the difference will be less than 1% normal acid, an amount which is usually considered the lowest limit for showing fermenta-

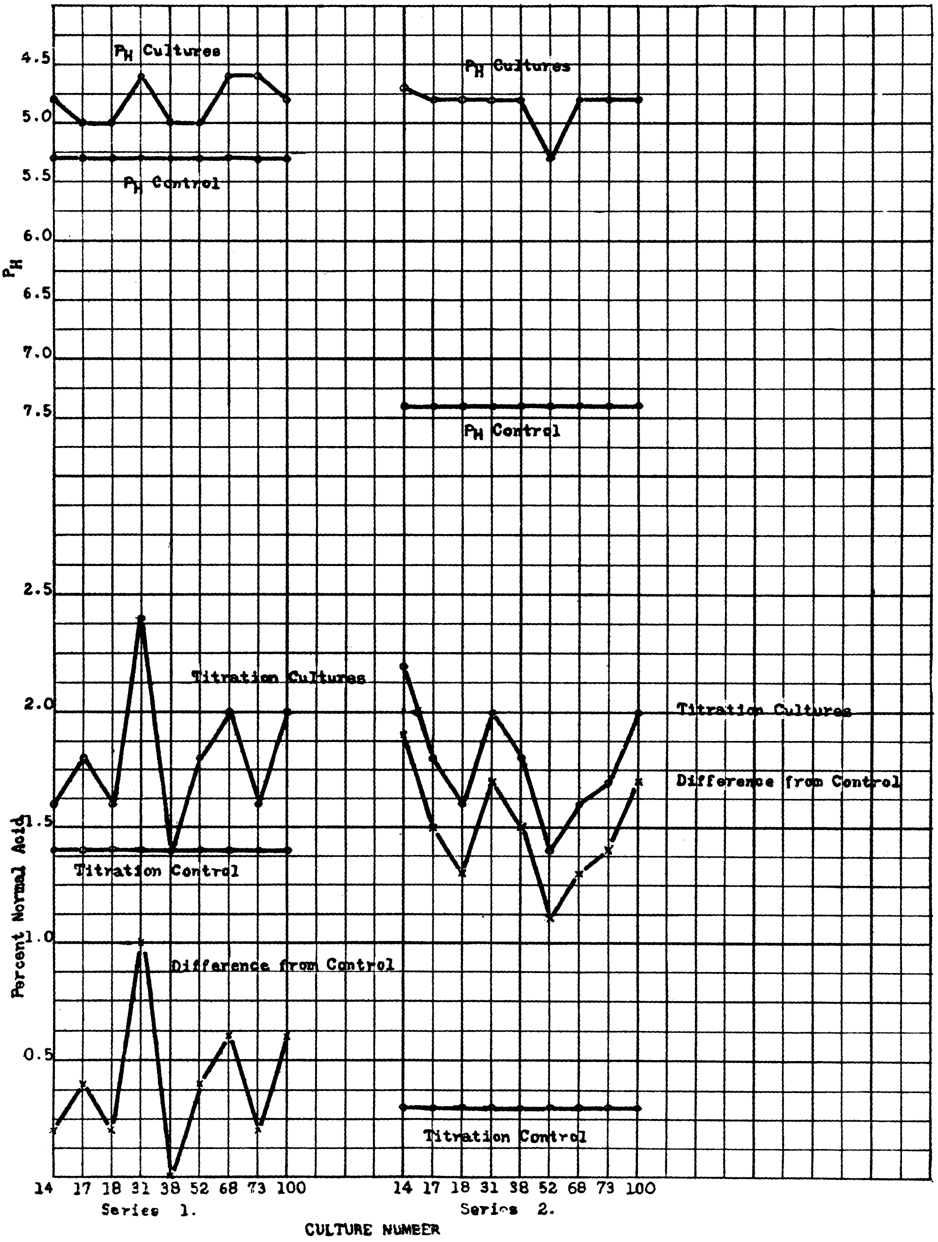

Chart 2.—Acidity as measured by titration and hydrogen ion concentration.

tion. This point is very clearly shown in Chart 2. A series of 9 cultures were inoculated into a dextrose-yeast-peptone medium and the reaction corrected to plus 1.4 (Fuller's scale) and the same cultures were inoculated into the same kind of broth having a reaction of plus 0.3 (Fuller's scale). After incubation for 7 days the cultures were titrated. The results are plotted at the bottom of Chart 2 where the curves represent the total titration, titration of the control, and the difference between the control and the total titration. On the left hand side of Chart 2 it will be observed that when the control titration of plus 1.4 was subtracted from the total titration the difference of per cent of normal acid formed was never above 1%. If, therefore, 1% of acid or less was considered negative fermentation then it would be said that none of the 9 cultures fermented dextrose. When the original reaction was plus 0.3 (Fuller's scale) and the control titration subtracted from the total titration and plotted as shown on the right hand side of Chart 2 it will be seen that the per cent. of acid formed in the same set of cultures was always above 1% acid. It would be considered, therefore, that these cultures fermented dextrose.

Based on titration methods there is a condition where with a high initial reaction the fermentation tests would be considered negative while with a low initial reaction they would be considered positive. When the series of cultures were inoculated into the medium of high and low reaction according to Fuller's scale duplicate sets were also inoculated and hydrogen ion concentration determined after 7 days' incubation. Results plotted at top of Chart 2 show that in the medium having an initial reaction of plus 1.4 (Fuller's scale) the hydrogen ion concentration of the control tubes was 5.3 and the cultures after incubation showed a limiting hydrogen ion concentration of from 4.6-5. In the other series where the original reaction was plus 0.3 the hydrogen ion concentration of the control tubes was 7.4 and after incubation it ranged from 4.7-5.4. It is apparent from this, therefore, that while by titration methods the cultures starting with a high reaction, that is plus 1.4 (Fuller's scale), would be said not to ferment dextrose, the hydrogen ion determinations would show that they fermented and reached their limiting hydrogen ion concentration the same as when their original reaction was at plus 0.3. In other words, the determination of hydrogen ion concentration would show a fermentation in both sets of mediums.

This point has been emphasized more perhaps than would at first seem warranted, but it is points of this nature that must be most carefully observed in the determination of the fermenting ability of bacteria.

SUMMARY AND CONCLUSIONS

The thermal death point of 27 strains of pathogenic streptococci from pathologic sources was never higher than 60 C. (140 F.) when heated in milk for 30 minutes. This fact, together with the results obtained by other investigators, seems to indicate that pathogenic streptococci are destroyed by the proper pasteurization of milk at 60 C. (140 F.) for a period of 30 minutes.

Some of eight cultures isolated from pathologic sources had thermal death point above 60 C. (140 F.) for 30 minutes and would, therefore, survive pasteurization. From their cultural characteristics we believe that these cultures were not pathogenic strains.

Our results indicate that in a dextrose-yeast-peptone medium, as used in our experiments, pathogenic streptococci reach a limiting hydrogen ion concentration from 5.4-6, while a nonpathogenic strain reached a higher concentration, namely, P_H 4.5-4.9. It is believed that this difference in limiting hydrogen ion concentration together with other characteristics such as the hemolytic power of the organism and the fermentation of carbohydrates and other test substances may serve as an important differential characteristic between pathogenic and nonpathogenic streptococci. Our results on this point are not at all conclusive and need confirmation by a study of a large number of cultures.

The value of the determination of hydrogen ion concentration in the study of fermentation cannot be too strongly emphasized and it is believed that titration methods for the determination of the fermentation of test substances should be discarded.